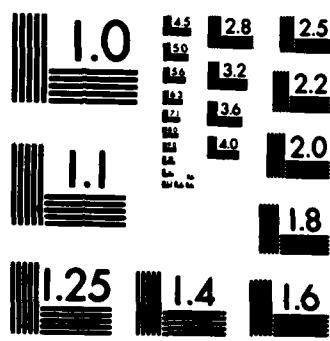


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1984 GORDON RESEARCH CONFERENCE  
ON CHEMISTRY AND BIOLOGY OF PEPTIDES

## **Final Report**

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(Conference Program Chairman)

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## Grant Report for the 1984 Gordon Conference on Chemistry and Biology of Peptides

The 1984 Gordon Conference on Chemistry and Biology of Peptides was held in Santa Barbara, California on February 5-10, 1984. The conference brought together scientists active in the fields of chemistry and biology of peptides as well as those researchers active at the interface of these fields. There were 150 participants; 92 from academia, 56 from industry, 2 from government; 120 from the United States and 30 from abroad. There was substantial representation by younger scientists reflecting the excitement in this field. The meeting was chaired by Drs. Johannes Meienhofer and Michael Rosenblatt. The grant was used to partially support attendance and participation by the foreign scientists, invited speakers, session chairpersons, and younger scientists.

The topics included the emerging biological importance of peptides as hormones, neurotransmitters and neuromodulators, enzyme substrates and other critically important biologically active substances. There was considerable exchange of scientific information both during the scientific sessions and informally by participants outside of the scheduled sessions. There were over 250 applications; only 150 of the applicants could be accommodated.

Below is a summary of highlights of the scientific presentations of the conference.

### Roger Guillemin - GROWTH HORMONE-RELEASING FACTOR (GRF).

The full sequence is thought to be 44 aminoacids in length. The smaller fragments found in tumor extracts appear to be extraction artifacts and not genuine biosynthetic products.

The rat GRF is very different from human. Guillemin has obtained monoclonal antibodies to native rat hypothalamic GRF. Administration of these antibodies blocks the endogenous growth hormone peaks, but does not block release of growth hormone by the pituitary which is stimulated by administration of rat 1-43-OH. The sequence of the human hypothalamic GRF is still being elucidated; however, the aminoacid analysis appears identical to the human tumor pancreatic GRF and co-elutes with it on HPLC. They have not found h-GRF in normal pancreas.

The structure of the gene encoding for prepro-hpGRF will be published shortly in PNAS by Gubler et al. There is a signal sequence followed by a cryptic peptide, followed by two arginines. This is followed by hpGRF, then glycine and arginine, followed by another cryptic peptide. The C-terminal cryptic peptide is present in large amounts in tumors and also in human hypothalamus.

GRF is homologous with PHI-27. The N-terminus is essential for biological activity, especially Tyr at position 1. However, 15-17 aminoacids can be deleted from the C-terminus with retention of full agonist properties, although there is an accompanying loss in potency.

Feedback appears to occur at the level of the pituitary. Rat GRF is more potent on rat tissue than human GRF. The mechanism of action appears to be through cyclic AMP. The case for c-AMP being involved is stronger for GRF than for any other hypothalamic releasing factor. Human GRF antibodies do not react with rat GRF but injected GRF attaches to rat GH secretory cells and then moves into the nucleus, i.e., the secretory granules of GH have a receptor for GHRH. GRF does not affect the secretion of newly synthesized (<3 hrs) hormone, only previously (> 12 hrs) synthesized hormone. This was shown through double-labeling experiments. Newly synthesized GH is not packaged and, therefore, lacks GHRH receptors. GRF has no effect on GH<sub>3</sub> cells because the latter can't make "old" (packaged) GH, only new GH which is released.

GRF may also have extra-hypothalamic and extra-pituitary effects. It releases calcitonin in neurotensin in medullary thyroid cancer cells. GRF is very specific for growth hormone release and does not affect prolactin or any other pituitary hormone secretion. Guillemin also feels that he has a promising binding assay for GRF based on a hormone analog. GRF is so potent at causing pituitary hormone release that the pituitary can be completely depleted of growth hormone within a few hours of exposure to GRF. This makes evaluation of the down-regulation phenomenon difficult.

One  $\mu$ g of GRF produces a maximum release of GH in a 70 kg human; the release is of 30-40 minutes duration. There is no point giving GRF again until 12 hrs later, when newly synthesized GH has been packaged.

Wylie Vale - CORTICOTROPIN-RELEASING FACTOR (CRF).

CRF is found more broadly in anatomy than GRF, which appears to be exclusively found in the hypothalamus.

The rat sequence for CRF is the same as human. CRF of sheep and rat differ significantly. CRF antagonists have now been made by Rivier, however, these are weak and have some intrinsic agonist activity.

A radioreceptor assay for CRF has been developed.

Glucocorticoids inhibit ACTH secretion at the level of the pituitary and the brain, but this inhibition is not complete. In Cushing's disease and depression, if CRF secretion is high and glucocorticoid levels are high, pituitary secretion will not be completely inhibited.

CRF stimulates the synthesis of mRNA for POMC (proopiomelanocortin). Glucocorticoids inhibit this synthesis.

CRF, when injected into the brain, inhibits pentagastrin induced acid secretion.

CRF and ADH are co-expressed in neurons and the release of both is inhibited by glucocorticoids. Pro-CRF and Pro-ADH are structure-related. Interestingly, ADH stimulates ACTH release by the pituitary, but inhibits hypothalamic CRF release. CRF may stimulate the sympathetic nervous system and inhibit the parasympathetic nervous system. Weaker secretagogues with a lower ceiling, such as vasopressin, catecholamines [via  $\alpha_1$  receptor], oxytocin and angiotensin II seem to augment the response to CRF.

A hepatoma cell line produces CRF. In this cell line phorbol esters produce an increase in CRF production.

Vale believes that antagonists will be more useful than antibodies in studying CRF effects in the CNS.

Vale speculated that CRF may be a key mediator of endocrine visceral and behavioral responses to stress.

Geoff Rosenfeld - DEVELOPMENTAL REGULATION OF NEUROENDOCRINE GENE EXPRESSION

The brain has one of the highest transcription rates in the body for the calcitonin

gene, one gene appears responsible for the synthesis of two mRNA's. The thyroid C-cell makes calcitonin and related cryptic peptides. The brain makes different products named the calcitonin gene-related product and other cryptic peptides. This may be a natural mechanism for increasing gene product diversity. The calcitonin gene-related peptide (CGRP) is found in motor, sensory, and vagal nerves and may be important in ingestive behavior. It is found in taste buds. It is also present in much of the endocrine system and may have potential effects on blood pressure. It may also be cooperative with calcitonin on bone and mineral metabolism, although this is completely speculative.

The mechanism for production of two different gene products is that the mRNA is cleaved after the entire message is made. Thus a tissue chooses to cut out the RNA encoding for either CGRP or CT. A given tissue must dictate a given poly A site selection for cleavage. There may then be promoters or inhibitors of this cleavage.

Peter Schiller - PEPTIDE HORMONE INHIBITORS

Peter Schiller reviewed the progress of design of inhibitors for multiple peptide hormone systems.

Victor Hruby - GLUCAGON ANTAGONISTS

Dr. Hruby pointed out that weak agonists often provide the first clue to antagonist design and emphasized the importance of good and diverse bioassays for antagonist design. The activation (message) region for glucagon is at the N-terminus. The binding region is C-terminal to this area. The synthetic sequence 1-6 alone has no intrinsic activity, presumably because it is unable to bind to the receptor.

Maurice Manning - ANTAGONISTS OF ADH AND OXYTOCIN

Dr. Manning reviewed already published work on design of antagonists for these systems.

T. G. Flynn - CARDIONATRIN

Dr. Flynn discussed the sequence of this compound and its relation to the atrial natriuretic factors. Seven nanomoles were sequenced. From the primary structure it appears that two  $\beta$ -turns may be present.

Marc Cantin - THE HEART IS AN ENDOCRINE ORGAN

Dr. Cantin discussed the 1-33 ANF. It is found only in the atria and not in the ventricles. Results with the Merck synthetic material suggest that the 8-33 fragment is as potent as the 1-33 fragment in terms of natriuresis and vasorelaxation using his numbering system.

Mary Napier - IDENTIFICATION OF THE ISOLATED ANF AS FREE C-TERMINAL ACID

Mary Napier reviewed the isolation and sequencing work done by the Merck group on ANF.

Steven Adams - ANF

Dr. Adams discussed the collaborative work by Monsanto and Dr. Needleman's group at Washington University. He discussed the three assays used: the intestinal smooth muscle assay, the vascular smooth muscle assay, and the natriuretic assay. He also discussed the probable existence of the high molecular weight precursor. The material they used was purified on Vydac columns. Compounds which were active in only the intestinal and natriuretic assay were 1-21, 2-21, 3-21, and 1-20. Compounds which were active in all three assays were 1-23 and 1-24. Hence, the sequence Ser-Phe-ARG-Tyr-OH appears to confer vascular activity to the rest of the structure. This talk was the first publication from the Needleman group which showed a C-terminal tyrosine for one of his peptides. This amino acid was found by all other laboratories as the C-terminus.

Rolf Geiger STATE OF ART OF SYNTHESIS

This was, for the most part, a good review lecture, but provided no novel concepts.

Geoffrey Tregear - WILL RECOMBINANT DNA REPLACE CHEMICAL SYNTHESIS?

Dr. Tregear reviewed the factors which determined whether or not rDNA rather than chemical synthesis should be considered. These include the size of the peptide, the need for D-amino acids or nonprotein constituents, convenience, efficiency-economy. Dr. Tregear pointed out that rDNA is very well suited to structure-activity studies. He reviewed some of the work performed in his laboratory and in collaboration with Genentech to make human relaxin by the rDNA approach. Both the natural gene and synthetic DNA were used. The chemical synthesis proved very difficult and there is little material available from natural sources. The issues they are facing in the rDNA approach include the efficiency of expression and the linkage of the disulfides bonds after biosynthesis. The gene was chemically synthesized by making stretches of synthetic oligonucleotides of unit length 50 or less and then ligating these together to form the gene.

Peptides made by the rDNA approach thus far include somatostatin, insulin, relaxin, angiotensin I, EGF, thymosin  $\alpha$ -I,  $\alpha$  and  $\gamma$ -interferon, secretin,  $\beta$ -urogastrone and others.

The rDNA approach also requires that the correct codons for the expression be selected, that the optimal choice of host cells and vector is made, that self-complementary sequences are avoided, and that useful restriction sites are incorporated.

Problems in the rDNA approach include the rapid degradation of peptide by bacteria, the need for a leader sequence in order to obtain secretion, and the problem that all sequences will have an N-terminal initiator methionine in place. In addition, bacteria can not put carbohydrate moieties onto peptides. Yeast can do so, but may do it incorrectly. Mammalian cells can place the correct carbohydrate onto the structure but tend to have poor expression.

A session on conformation and drug design involved presentations by Hassall (Roche, U.K.), Mazur and Clare (Searle) and Smith (Upjohn), and by Jim Snyder (Merck). It was agreed that the Merck paper was the best of that session and the most thoughtful. Those not familiar with the issues probably did not get a balanced overview of the techniques from the session.

**Joel Habener - STRUCTURE AND EXPRESSION OF GLUCAGON GENES**

Joel Habener reviewed the glucagon family of peptides which includes glucagon, GRF, secretin, VIP, GIP, glicentin, PHI.

**David Meyer - COTRANSLATIONAL EVENTS IN PEPTIDE SECRETION**

Dr. Meyer reviewed the current state of knowledge on the signal recognition particle which is made of six subunits which total a molecular weight of 250,000 and a 7s RNA. He also reviewed the interaction of the docking protein with the signal recognition particle (SRP). Apparently the wheat germ system does not contain SRP; however, the rabbit reticulocyte lysate system contains SRP in the cytoplasm, not on the membrane. In addition, the cytoplasm of dog pancreatic membranes does contain SRP. Thus far no bacterial SRP has been identified. SRP can be washed off the membrane with salt. Salt washed reticulocyte lysate will pick up SRP from cytoplasm. Rough microsomes have SRP which can be washed off leaving a docking protein which can be cleaved with elastase/salt, a reversible operation. The docking protein is the SRP receptor.

**Edward Herbert - GENERATION OF DIVERSITY IN NEUROPEPTIDE GENE EXPRESSION**

Dr. Herbert revealed that there may be 35 to 40 kallikrein genes present in the neuronal areas responsible for the biosynthesis of endogenous opioids. He believes that these enzymes may be responsible for processing of the opioid peptide precursors. Different sets of protein processing enzymes are expressed in different tissues. Adrenalectomy produces an increase in mRNA for POMC in the anterior lobe; glucocorticoids block this effect.

**Janet Kurjan - STRUCTURE AND FUNCTION OF YEAST  $\alpha$ -FACTOR GENES**

Dr. Kurjan reviewed the gene structure of the alpha factor which is a 13-amino acid pheromone. The alpha factors are biosynthesized as an N-glycosylated precursor which induces cell surface agglutinins on yeast, important in yeast reproduction.

**Janakiraman Ramachandran - PHOTOAFFINITY LIGANDS OF ACTH**

Dr. Ramachandran reviewed the synthesis of a photoaffinity ligand for ACTH. An analog of ACTH was used in which the methionine was substituted with norleucine. In addition, a photolabile group was attached to tryptophan. Photoaffinity labeling of adrenocortical cells leads to irreversible activation. He has also found that calcium is needed for hormone binding and continued occupancy of the receptor (but not calcium) is needed for steroid generation. Low temperature enhances binding rather than diminishing it. Much higher concentrations of ACTH are needed to saturate lipolysis than steroidogenesis.

**Ora Rosen - THE INSULIN RECEPTOR**

Dr. Rosen reviewed the structure of the insulin receptor, which is made of 4 glycosylated subunits. These are biosynthesized as a single large precursor. The insulin receptor autophosphorylates on tyrosine, which may be the first step in hormone action. However, evidence for the significance of this step in correlation with other metabolic events within the cell has yet to be obtained. The autophosphorylation is an intramolecular

reaction and two phosphates are incorporated per receptor. Tyrosine phosphorylation may be involved in normal and abnormal growth regulation. It may play an important role, for example, in the clustering of receptors rather than in the mechanism of action. We need inhibitors to determine the importance of tyrosine protein kinases.

Russell Ross - PLATELET-DERIVED GROWTH FACTOR (PDGF)

PDGF may be a mitogen. It may also play a role in atherosclerosis. PDGF binds partially to the EGF receptor. When both receptors are present, binding of PDGF to its receptor may affect binding of EGF to its receptor, but not vice-versa. PDGF after binding increases phosphatidyl inositol levels leading to increased levels of diacylglycerol. It is a chemoattractant for smooth muscle cells and fibroblasts. PDGF may also work through autophosphorylation. PDGF may induce cholesterol synthesis.

James Staros - EGF

The receptor for EGF is also a glycoprotein; it has no subunits - it is a single polypeptide. He has labeled the kinase site with a radiolabeled analog of cyclic AMP.

Michael Sela - SYNTHETIC PEPTIDES AS ANTIGENS FOR VACCINES

In general, the most exposed region appears to be the immunogenic. He discussed the "active" ingredient in Freund's adjuvant, which is N-acetyl muramyl 1-alanyl-D-isoglutamine. He also discussed a strategy for raising antibodies to two important antigens such as cholera and tetanus toxoid by attaching cholera toxin fragments to tetanus toxoid. One saw tolerance to tetanus thus this approach does not appear useful.

Edwin Beachey - PROTECTIVE IMMUNITY BY SYNTHETIC PEPTIDES OF STREPTOCOCUS

Dr. Beachey described efforts to generate a vaccine that would be protective for rheumatic fever and rheumatic heart disease. The difficulty is to get antibodies which will react with the M-protein portion of the bacteria but will not cross react with heart tissue. If the latter occurs, then one may induce rather than protect against rheumatic fever. He attached his synthetic peptides to polylysine and got good opsonizing antisera as well as monoclonals. None of the synthetic peptides caused cross-reaction with heart epitopes.

Christian Birr described work with synthetic antigenic determinants specific for the phosphorylation sites of the cell transforming protein pp60<sup>src</sup> from Rous Sarcoma Virus.

Lila Gierasch and Betty Eipper/Dick Maine received the newly established du Vigneaud Awards for scientists under 40 years of age.

Bruce Erickson discussed synthetic lactam and thiolactone models of protein metastable binding sites. This material was the subject of a recent lecture in Rahway.

Herbert Weingartner - PEPTIDES AND COGNITION

Dr. Weingartner pointed out that many of the assays used for pharmacology of behavior become the focus of the studies and don't necessarily reflect general behavior. For

instance, is learning to run a maze the same as learning to avoid a shock? He described different kinds of memory such as episodic vs. knowledge memory. He discussed differences in memory in Alzheimer's disease vs. Korsakoff's syndrome and Huntington's disease. The latter two syndromes display decreased learning, but patients can learn with repetition. In Alzheimer's disease, there is no learning with repetition; there is no encoding of information.

Gerard Smith - CHOLECYSTOKININ (CCK) AND FEEDING

Dr. Smith reviewed the use of CCK-8 in inhibiting the hyperphagia of sham-feeding. He believes that this kind of assay (in which animals have a draining gastric fistula) is better than less rigorous assays of appetite. He believes that CCK-8 causes a "normal" type of satiety. The sulfate on tyrosine is needed for satiety activity.

He believes that CCK acts through peripheral neuronal structures that go to the brain. If CCK is given intraperitoneally at a dose of 2  $\mu\text{g}/\text{kg}$ , early satiety is observed even after sham feeding. On the other hand peptides such as gastrin, secretion, glucagon, insulin, neuropeptides, etc., show effects in feeding but not in sham feeding experiments. He believes that this is mediated by CCK interaction with the vagus nerve and its afferents to the brain via its gastric branches. No effect of CCK is lost when the efferent vagus fibers are abolished. Sulfated CCK is inactive. Satiety effects parallel the other CCK S/A relationships.

Bombesin is the only other peptide that inhibits sham-feeding. He believes that bombesin may act through the entire visceral nervous system.

He discussed human studies in which 9 of 12 humans had a 15% inhibition of their caloric intake when given CCK-8 intravenously. He cited confirming experiments by Stacker in 1982.

He now has evidence that chronic treatment with CCK in rats prior to meals leads to weight reduction.

He also cited studies in three human subjects in which no side effects were seen.

Howard Judd discussed the use of LHRH agonists in gynecological disease.

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